

REMARKS

STATUS OF THE CASE

In the Office Action issued November 30, 2007, all of the claims were rejected under 35 U.S.C. §112, first paragraph. In this Amendment, Applicants traverse all rejections. Claims 43-47 and 50-54 are also cancelled herein without prejudice and without disclaimer of the subject matter therein. Claims 1-42, 48 and 49 were cancelled previously. Claims 55-69 are new, and find support in the claims and specification as originally filed. Upon entry of this Amendment, Claims 55-69 are currently pending. The Examiner is respectfully requested to reconsider and withdraw the rejection of record, and to allow all new claims accordingly, in view of the amendments and remarks contained herein.

SPECIFICATION AMENDMENT

The specification is amended to correct a typographical error. No new matter is added.

APPLICANTS' CLAIMS COMPLY WITH 35 U.S.C. §112 1ST PARAGRAPH

In the November 30, 2007 Office Action, Claims 43-47 and 50-54 stand rejected under 35 U.S.C. §112, first paragraph, for allegedly failing to reasonably provide enablement for a method of treating hepatocarcinoma, mesenchymal tumors, neuroectodermal cancer, Ewing's sarcoma and malignant hemopathies in a patient comprising administering a therapeutically effective amount of a composition comprising cDNA of a zyxing gene fragment thereof or a complementary sequence. Further, the Office Action asserts that the specification does not enable any person of ordinary skill in

the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with these claims. (Office Action at page 3). This rejection is respectfully traversed.

At the outset, Applicants thank the Examiner for finding that the specification is enabling for a method of treating B16F10 murine melanoma tumor cells in a patient comprising administering a therapeutically effective amount of a composition comprising a cDNA of a zyxin gene. Nevertheless, Applicants submit that all claims are fully enabled.

The bases for the §112 rejection are perhaps best summarized in the Examiner's response to Applicants' arguments. On page 10 of the Action, the Examiner states:

"First, the Examiner acknowledges and concedes that the Declaration by Celine Bouguet shows the *in vivo* efficacy of intratumoral injection of a recombinant adenovirus, e.g., AdZyxine, coding for the zyxine gene in Swiss nude mice inoculated with B16F10 murine melanoma cells. However, the Examiner recognizes that this showing in a mouse model using one type of cancer, does not appear to be commensurate in scope with the claimed invention since the claims broadly encompass a method of treating any and/or all hepatocarcinomas, mesenchymal tumors, neuroectodermal cancer, Ewing's sarcoma and malignant hemopathies in any patient including humans. In the instant case, the Examiner recognizes that the specification does not contain any teachings that address the ability of the composition to treat a human subject or even its ability to work *in vivo*. Specifically, the specification has not taught an appropriate tested dose for humans, the amount of a nucleic acid molecule comprising cDNA of a zyxin gene, a fragment thereof or a complementary sequence necessary for successful treatment, the number of cells to be treated, the number of times the treatment needs to be administered or the most appropriate route of administration. Thus, in view of the state of the art as described above, the specification appears to be silent on any of these critical aspects of gene therapy."

In essence, the Examiner appears to allege two main points.

- The specification in general, and the Declaration of Dr. Bouguet in particular, does not establish that methods of treatment were enabled at the

time of filing commensurate in scope with the claimed treatments of cancer types in humans.

- The specification does not teach specifics regarding treatment regimens. In particular, the specification does not enable gene therapy methods.

As discussed below, Applicants respectfully submit the Examiner's analysis is incorrect on both points.

I. THE BREADTH OF THE CLAIMS ARE FULLY ENABLED AS TO ALL SUBJECT CANCERS TREATED

The present invention, as amended herein, is directed to a method of treating cancers having a zyxin gene expression/abnormality selected from the group consisting of hepatocarcinomas, mesenchymal tumors, neuroectodermal cancer, Ewing's sarcoma, melanoma, and malignant hemopathies associated with chromosomal anomalies of region of 7q34/q35 of zyxin gene. These cancers are treated by actin network stabilizing active agents comprising, for example, a zyxin-related protein or nucleic acid molecule specific to such cancers. Claims 62-69 are even further directed to methods where the presence of such a tumor underexpressing a zyxin gene is identified during the treatment. As such, the scope of the claims, and in particular, the treatable cancers enumerated in the amended claims, have been focused to include only those cancers that have been shown to have zyxin gene expression/abnormality, such as those disclosed in the specification and presented in the amended claims. See the Specification as filed at page 13, par. [0053]. Thus, the methods do not purport to treat any and all hepatocarcinomas, mesenchymal tumors, neuroectodermal cancers, etc.

Applicants also respectfully note that issues relating to the enablement of claims requiring prevention of the various cancers using the pharmaceutical compositions

described are no longer relevant. Applicants amended the claims to remove the “prevention” claim limitation in the August 7, 2007 Amendment. Applicants thus respectfully submit that the application as filed provides adequate guidance to those of ordinary skill in the art to practice the full scope of the claims.

Underlying the Examiner’s analysis is an assessment that reduction to practice of Applicants’ methods would require undue experimentation. To support this assessment, the Examiner points to complexity of the art, and alleged failures by others in practicing certain treatment methods (e.g., gene therapies). Applicants respectfully point out clear prescription in MPEP 2164.01 that a relevant factor in assessing enablement is not particularly the amount or complexity of experimentation required to practice an invention, but whether the experimentation required is routine:

The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. MPEP §2164.01 (citations omitted).

In this light, the present specification teaches how to make and use the invention without undue experimentation.

To evidence enablement, Applicants have previously provided evidence in the form of Declarations by Drs. Celine Bouquet and Michel Jean Robert Perricaudet supporting that one of ordinary skill in the art, at the priority date of the present application, would not have had undue difficulty in treating at least two distinct forms of cancer *in vivo* with the pharmaceutical compositions of the present application. The Examiner has acknowledged the showings made, but maintains that the Declarations do not evidence enablement of the entire scope of claims. Applicants respectfully disagree.

The Declarations describe the replication of the antitumor effects of Adenoviral constructs comprising an E1-E3 deficient Adenovirus harboring a zyxin gene under the

control of a cytomegalovirus (CMV) promoter in mice. (See Specification as originally filed, page 13, par. [0051-0052]. As can be seen from the results replicated by Dr. Bouquet, the reduction of two distinct tumors *in vivo* in accepted and known cancer mice models provides that the claims were enabled as of the date of filing and in particular for the new claims presented herein. The first tumor, B16F10, was suppressed by 73% - 79% *in vivo* after a single injection intratumorally with 2×10^9 pfu of recombinant AdZyxine. B16F10 is a well known and highly experimented murine melanoma cell line. Applicants further provide publications relating to the use of the B16F10 murine melanoma cell line as an *in vivo* experimental model for the testing and efficacy of a GM-CSF secreting tumor cell vaccine. Kayaga, J et al., "*Anti-tumour activity against B16-F10 melanoma with a GM-CSF secreting allogeneic cell vaccine*" Gene Therapy (1999) 6:1475-1481. A copy of the reference is attached. In addition, the B16F10 murine melanoma cell line has been used to support the enablement of several issued patents for example, U.S. Patent No. 4,793,986, Serino et al., issued December 1988 entitled: "Macromolecular Platinum Antitumor Compounds" and U.S. Patent No. 5,880,129, Kohn et al., issued March 1999 entitled: "Methods of Inhibiting Invasion and Metastasis of Malignant Solid Tumors."

The second tumor suppressed in Dr. Bouquet's AdZyxine recombinant virus challenge was the NIH 3T3 EF cell line. This cell line used by Dr. Bouquet expresses the fusion protein EWS-FLI-1 which is responsible for the cancer Ewing's sarcoma. Specifically, the human FLI-1 gene is rearranged in the majority of cases of Ewing's sarcoma and neuroectodermal tumors that share t(11;22)(q24;q12) chromosome translocations. Please see Ohno, T, et al., "*EWS/Fli-1 Chimeric Protein Is a Transcriptional Activator*", (1993) Cancer Res., 53:5859-5863. A copy of the Abstract is

attached. This fibroblast tumor model was suppressed by 57%-66% as compared to non-zyxin containing adenovirus AdCO1 and PBS control respectively. The NIH 3T3 cell line is an extremely well known and tested cell line, and has been in continuous use as a fibroblast tumor cell since the late 1960's, Jainchill J.L., et al., "Murine sarcoma and leukemia viruses: assay using clonal lines of contact-inhibited mouse cells." J. Virol. 1969, 4(5):549-53. These cells were established originally from mouse embryo cultures and widely used as a target for oncogene mediated transformation, undergoes spontaneous morphological transformation at a high rate under conditions in which certain physiological constraints on growth are introduced. See Rubin, A.L., et al., "Physiological induction and reversal of focus formation and tumorigenicity in NIH 3T3 cells" Proc. Natl. Acad. Sci., (1990), 87:10005-10009. A copy of the reference is attached.

Hence, the *in vitro* results shown in the Specification at pages 35-38, evidenced by the *in vivo* results accordingly obtained by Dr. Bouquet with respect to suppression of the EWS-FLI-1 oncogenicity and melanoma tumors in mice establish that the specification provides enablement for the full scope of the claims herein. Moreover, given that these experiments provide direct evidence of efficacy against EWS/FLI-1 containing cancers, Applicants respectfully assert that claims relating to neuroectodermal cancer and Ewing's sarcoma are at least enabled by the present application.

The Office Action also appears to raise issues related to the lack of examples in the Specification as filed regarding the *in vivo* efficacy of a nucleic acid molecule comprising cDNA of a zyxin gene, a fragment thereof, or a complementary sequence. The Office Action alleges that the Specification does not show any success in treating a disease by using the pharmaceutical compositions of the claims.

Applicants respectfully traverse the Action's assertion that the Specification provides no examples of *in vivo* efficacy. Specifically, the Specification provides *in vivo* results in mice describing the protective effects of over-expression of zyxin and the induction of a loss of malignant phenotype in the nude mouse. There is clearly a reduction in the development of tumors after subcutaneous injection of the various cell lines described in the Material and Methods section, in particular, page 19, par. [0075-0079] and par. [0085] and discussed in the results section on page 23, par. [0087], par. [0099-0105], and in particular, Tables 1 and 2 on pages 27 and 29 respectively.

Furthermore, given the guidance and working examples in the Specification as filed, Dr. Bouquet was able to independently replicate the effectiveness of the exemplified pharmaceutical compositions against two distinct tumors, i.e. melanoma and a representative cancer of Ewing's sarcoma using viral constructs encoding a nucleic acid molecule encoding zyxin. Therefore, the evidence provided in the Specification of *in vivo* efficacy using a nucleic acid molecule to treat two distinct cancers that have a zyxin gene expression/abnormality rebuts the Office Action's assertion that the specification lacks any success in treating a cancer disease as presently claimed using a nucleic acid comprising cDNA of a zyxin gene, a fragment thereof or a complementary sequence thereof.

Furthermore, Applicants respectfully submit that a showing of *in vivo* human efficacy is not required to render the claims enabled. Applicants have enabled the full scope of the amended claims by showing a correlation between *in vitro* and *in vivo* mouse models and the claimed method of use. As noted in section I, the pharmaceutical compositions and their methods of use in various *in vivo* tumorigenicity mouse models have shown to provide protection against different tumor types. The mouse models and tumors induced in the mice

are sufficiently correlative to constitute a working example for human efficacy, in the claims as amended. Cf., MPEP § 2164.02. Moreover, the claims as amended are limited to those tumors having zyxin gene expression/abnormality and not any hepatocarcinoma, mesenchymal tumors, neuroectodermal cancer, Ewing's sarcoma and malignant hemopathies. The Specification as filed thus provides adequate examples and guidance in how to identify cells having a zyxin gene expression abnormality using anti-zyxin antibodies in Western Blots and RNA probes in Northern Blots.

II. THE CLAIMED TREATMENT REGIMENS ARE FULLY ENABLED

On page 10 of the Office Action, the Examiner alleges, "... the specification has not taught an appropriate tested dose for humans, the amount of nucleic acid molecule comprising cDNA of a zyxin gene, a fragment thereof or a complementary sequence necessary for successful treatment, the number of cells to be treated, the number of times the treatment needs to be administered or the most appropriate route of administration." Applicants submit that the specification provides ample guidance to one of skill in the art to determine appropriate regimens to practice the claimed methods. In this regard, the MPEP states:

[I]t is not necessary to specify the dosage or method of use if it is known to one skilled in the art that such information could be obtained without undue experimentation. If one skilled in the art, based on knowledge of compounds having similar physiological or biological activity, would be able to discern an appropriate dosage or method of use without undue experimentation, this would be sufficient to satisfy 35 U.S.C. 112, first paragraph. MPEP § 2164.01(c).

Applicants submit that undue experimentation is not needed to practice the claimed methods.

In particular, while Applicants acknowledge the Examiner's concern relating to

the large amount of experimentation required in the field of gene therapy for cancer, this factor is not probative for the conclusion that the amended claims cannot be practiced without undue experimentation. Again, as noted above, the amount or complexity of experimentation is not dispositive of whether experimentation is “undue,” particularly if the art typically engages in such experimentation. Furthermore, a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.” See, MPEP §2164.06.

Prior to the time of filing the present application there were many completed and ongoing cancer gene therapy trials involving viral constructs encoding a therapeutic gene targeting one or more cancers. Applicants respectfully submit that the ability to formulate specific pharmaceutical compositions and use them in the claimed methods, including phase I clinical trials, was well established at the time of filing, and a matter of routine experimentation. Moreover, selection of viral vectors, their promoters and methods of administration are provided in detail in the specification (for example, the generation of retroviral stocks having a human zyxin gene inserted and under the control of a CMV promoter is described in detail in the Material and Methods section of the application on pages 17-23. Methods for determining the expression of zyxin in various cell types can also be found in the Material and Methods section of the application.

Applicants take notice of the literature the Examiner has presented to support the contention that the methods of treating disease with gene therapy was unpredictable at the time of filing. However, in rebuttal, Applicants note that at the time many of the cited references were published, gene therapy was a relatively nascent technology when compared to the date of filing of the present application. As an illustrative example, Verma

et al. discloses “Thus far, the problem has been an inability to deliver genes efficiently and to obtain sustained expression.” The Office Action states the same issue. Applicants assert that in the context of cancer gene therapy, issues relating to delivery and sustained expression have been largely overcome by intratumoral administration rather than systemic administration for example by intravenous routes. For example, several well represented cancer gene therapy trials have administered the viral vector through intratumoral administration with the aid of catheterization for injection targeting to the tumor. See for example Khuri, F.R. et al., A controlled trial of intratumoral ONYX-015, a selectively replicating adenovirus, in combination with cisplatin and 5- fluorouracil in patients with recurrent head and neck cancer.” Nature Medicine, (2000), 6(8):879-885, a copy of which is attached.

Moreover, the development of viral strains, in particular, adenoviral strains having gene-deletion mutants, such as in the E1B-55kDa region that enables the virus to infect p53 negative tumor cells preferentially over p53+ wild type cells, were well known at the time the present application was filed. Again similar issues relating to gene delivery are raised in Eck et al., which can be overcome by applying intratumoral administration and selection of oncolytic viral strains that have a preference for tumor cells either through cell surface receptors selective for a particular tumor type, or viral vectors having gene deletion mutations that render the gene therapy viral strain preferentially infectious towards the tumor cell.

Rubanyi is cited as a reference which allegedly teaches that problems identified in Verma et al., Ross and Eck et al., have remained unsolved. However, Applicants view Rubanyi as providing numerous examples where obstacles relating to gene delivery have been overcome. For example, on page 122, Rubanyi states, “A variety of physical gene

delivery methods have been introduced to achieve better local tissue targeting of vectors. An example of the effective physical targeting is catheter-mediated gene transfer to various regions of the circulation... ." With regards to biological targeting, Rubanyi states on page 123, "For example, adenoviral vectors, which contain polylysine motifs or RGD genetically incorporated into the fiber, have been shown to enhance the transduction of a variety of cells which lack adenovirus (CAR) receptors (Wickham, 2000)."

Applicants further note several articles published prior to the date of filing the present application that discuss advancement in the treatment of incurable cancers using viral mediated gene therapy. For example, Khuri, F.R. et al., describes the successful treatment of squamous cell carcinoma using Adenoviral vector ONYX-015 which, like the AdZyxine viral vector used by Dr. Bouquet, lacks E1 and E3 gene regions, in particular the E1B gene. Methods described in the ONYX-015 trial are standardized Adenoviral dosages and can be obtained using routine experimentation. In addition, U.S. Patent Nos. 5,677,178, 5,801,029, 6,296,845 and 6,726,907 all relate to patented Adenoviral gene therapy compositions and describe methods of their use. Methods of administration, tested dosages for human applications and preparation of titers were disclosed in the above referenced patents, and were available to one of ordinary skill in the art prior to the filing of the present application.

In Khuri, et al. *supra*, the, doses and routes of administration were 1×10^{10} plaque forming units, delivered intratumorally, *i.e.* injected into or at the periphery of the tumor. Similar titers and identical administration methods are described in Schuler, M., et al., J. Clin. Oncology, (2001), 19(6): 1750-1758 (7.5×10^{12} particles), citing Schuler, M., et al., Human Gene Ther. (1998), 9:2075-2082). In both of these cancer gene therapy trials, the patients treated with the Adenoviral vector did not experience adverse toxicities and

reductions in tumor sizes were observed in comparison to treatments without the viral medicated gene therapy.

Hence, the patient dosages, the amount of nucleic acid used per treatment, the patient criteria, routes of administration, or the number of times the treatment needs to be administered can be derived using routine experimentation and are taught in several phase I and II clinical trials using viral constructs which are exemplified in the present invention. For example, from the Adenoviral cancer gene therapy references cited herein, one of ordinary skill in the art can tailor a treatment for the subject cancers having a zyxin gene expression/abnormality with an Adenoviral vector comprising an AdZyxine Ad vector delivered intratumorally, with dosages that can include 1×10^{10} to 1×10^{12} particles/or p.f.u. per dose administered 1-5 times over a period of 10 days. Although this is an example derived from the available literature, one of ordinary skill in the art could conduct safety trials to determine the maximum dosage levels having a commensurate benefit to risk ratio, while also following national and state protocols for gene therapy trials.

CONCLUSION

Applicants submit that the claimed methods of treatment are not unduly broad – they are focused on specific types of cancer and are treated with active agents that Applicants have discerned are specific to those types of cancer. Practice of these methods does not require undue experimentation, beyond the level of dosage formulation and preclinical and clinical studies routine in the art. Gene therapies, in particular, were well understood to have utility, and design of such therapies was well within the skill of the ordinary artisan at the time of filing. Accordingly, Applicants submit that the claims are fully enabled, and request withdrawal of the rejections under 38 U.S.C. §112.

It is believed that a full and complete response has been made to the outstanding Office Action and the present application is in condition for allowance. If the Examiner believes that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (248) 641-1600.

Respectfully submitted,

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